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Characterization of milkweed (*Asclepias* spp.) seed proteins

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ABSTRACT

Milkweed (*Asclepias* spp.) is a crop grown mainly for the production of floss used as hypoallergenic fillers in comforters and pillows. The seeds end up as by-products. Milkweed seed contains 21% oil and 30% crude protein (dry basis). The oil is similar in quality to soybean oil, but there is no information on the properties of milkweed protein. This study determined the MW of major fractions, soluble classes, amino acid composition, and functional properties of milkweed seed protein. Ground milkweed seeds were analyzed for proximate composition and amino acid profile, as well as, subjected to SDS-PAGE and protein functionality tests. Reduced proteins showed eight distinct bands with MW ranging from 6.5 to 59.3 kDa. The dominant protein classes were water-soluble (22%) and salt-soluble (15%). Solubility of milkweed seed protein was lowest (12%) at pH 4, 40% at pH 7, and reached a maximum (60%) at pH 10. The protein produced substantial foam volumes, but foam stability was poor. Its emulsifying capacity was excellent, especially at pH 10, and emulsions formed were stable. Water-holding capacity and surface hydrophobicity index values were higher at pH 7 than at pH 10. These results showed that milkweed seed protein has functional properties that may find use as a thickener, protein extender in adhesives, or emulsifier in paints.

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1. Introduction

Milkweed (*Asclepias* spp.) is a perennial plant that grows abundantly in regions where maize is cultivated in the United States (Knudsen and Zeller, 1993). The common milkweed (*Asclepias syriaca*) is familiar to most people as the dominant food source for monarch butterflies. The plant and seeds contain cardiac glycosides (cardenolides) that are essential for the growth and survival of the monarch larvae (Malcolm and Zalucki, 1999).

Milkweed is also an industrial crop. The seed pods are harvested for their floss, which has been found to provide high thermal insulation (Crews et al., 1991). Since 1989, Natural Fiber Corp. (Ogallala, NE) has produced milkweed seed floss commercially as hypoallergenic fiber fillers in comforters and pillows (Knudsen and Zeller, 1993).

Milkweed seeds end up as by-products of floss production and have limited applications as plants for landscaping and erosion control. Seeds contain 21% oil and 32% crude protein (dry basis) (Evangelista, 2007). Holser (2003) produced refined and bleached milkweed seed oil that consisted of predominately unsaturated fatty acids (34% oleic, 50% linoleic, 1% linolenic) and had very low oxidative stability, but the oil could be used as an alternative triglyceride source. The meal is

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not suitable for animal feeds because the cardenolides cause cardiac problems; however, the defatted seed meal has been shown to kill nematodes and field worms when used as soil amendment (Harry-O'Kuru et al., 1999).

Currently, there is no information on the properties of milkweed seed protein. These properties should be examined to identify other possible value-added uses of the seed protein. The present study was conducted to determine some basic chemical and functional properties of protein in milkweed seed. These properties include molecular weights of major fractions, soluble classes, amino acid composition, and functional properties, such as solubility, surface hydrophobicity, foaming, emulsification, and water-holding capacity.

2. Materials and methods

2.1. Starting materials

Whole milkweed seeds were provided by Natural Fiber Corp. (Ogallala, NE). Seeds were cleaned by screening and aspiration. Cleaned whole seeds were then ground into ca. 30-mesh particle size by using a Cuisinart coffee grinder (Model DCG-12BC, East Windsor, NJ) for 2 min. Samples were stored in capped vials at room temperature for analyses.

2.2. Proximate analyses

Moisture, crude protein (%N × 6.25), and crude oil contents of the samples were determined by using AOCS standard methods Ba 2a-38, Ba 4e-93, and Ba 3–38, respectively (AOCS, 1998).

2.3. Determination of soluble classes

The method of Hu and Esen (1981) was used to determine four major soluble classes of protein. Ground milkweed seed was subjected to sequential extraction by cold water (30 mL:1 g), 0.5 M NaCl (30 mL:1 g), 70% ethanol (20 mL:1 g), and 0.1 M NaOH (25 mL:1 g). After each extraction cycle, the mixture was centrifuged for 15 min at 30,000 × *g*. The supernatant was collected for protein content determination while the solid residue was used for the next extraction. Amounts of soluble protein were determined spectrophotometrically using the Biuret method. The amount of protein remaining in the spent solids was determined by combustion (LECO FP-528 Protein/Nitrogen Determinator, Model 601-500, St. Joseph, MI) using AOCS method Ba 4e-93 (AOCS, 1998).

2.4. Amino acid analysis

Amino acid profiles of ground, defatted milkweed seed, water-soluble protein fraction, NaCl-soluble protein fraction, and residue after extraction (spent solids) were determined exactly as described previously by Wu and Hojilla-Evangelista (2005). Hydrolysates were prepared by using 6N HCl for 4 h at 145 °C and the amino acids were then determined by using cation exchange chromatography. For methionine and cystine determination, samples were first oxidized by performic acid before hydrolysis. Tryptophan was determined by a colorimetric method following enzymatic hydrolysis by pronase. Duplicate determinations were done for each hydrolysate.

2.5. Gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done by following the method of Wu and Hojilla-Evangelista (2005). Ground milkweed seeds were weighed out to provide 4 mg protein/mL in 500 μL of sample buffer composed of 42 nM Tris–HCl (pH 6.8), 2% SDS, 7% glycerol, 4.4% β-mercaptoethanol, and 5 M urea. The mixture was then heated in a boiling-water bath for 5 min. Protein samples (15 μL) were loaded onto 4–12% Bis–Tris NuPAGE precast gradient gel (Invitrogen Corp., Carlsbad, CA). Bio-Rad (Bio-Rad Laboratories, Hercules, CA) pre-stained broad range SDS-PAGE protein standards (6.5–196 kDa) were included in the gel. Electrophoresis was done in a Novex XCell II Mini Cell system (Novex, San Diego, CA) and using the NuPAGE MES-SDS running buffer (SDS, Tris, and 4-morpholinoethane sulfonic acid).

2.6. Functionality tests

Ground milkweed seeds were prepared for protein functionality testing by following the method that we used for *Cuphea* (Hojilla-Evangelista and Evangelista, 2006), which involved defatting at 25 °C using hexane. This method allowed us to evaluate the properties of the protein in as close to its native state as possible (because no heat was applied) and was also found to adequately eliminate the influence of oils on functional properties of protein as long as the residual oil content was ≤0.5% (db). Six extraction cycles were done to obtain the desired residual oil content in the meal. In each extraction, the mixture (1 g sample:10 mL solvent) was stirred for 1 h with a magnetic bar. The mixture was allowed to stand until the supernatant has cleared, and then the solvent layer was pipetted out and discarded. Defatted ground samples were air-dried in a fume hood until the hexane smell was no longer detectable, and then stored in screw-capped polyethylene containers at room temperature until use.

2.6.1. Protein solubility

Protein solubilities of samples containing 10 mg protein/mL were determined at pH 2.0, 4.0, 5.5, 7.0, 8.5, and 10.0 according to the method of Balmaceda et al. (1984). The amounts of soluble protein in the supernatants were determined spectrophotometrically using the Biuret method. Bovine serum albumin was the protein used to generate the standard curve.

2.6.2. Surface hydrophobicity

Surface hydrophobicity indices (S_0) of soluble proteins in the extracts were determined at pH 7.0 and 10.0 as described by Hojilla-Evangelista et al. (2004), which was adapted from the method of Sorgentini et al. (1995). Samples were weighed out to provide 2 mg protein/mL and dispersed in 0.01 M phosphate buffer (pH 7) or 0.0249 M $NaHCO_3$·Na_2CO_3 buffer (pH 10). Supernatants were diluted with the pH 7 or pH 10 buffer to yield 1/5, 1/10, 1/50, 1/100, and 1/500 concentrations of the starting protein content. The fluorescence probe was 8.0 mM

8-anilino-1-naphthalene sulfonate (ANS). Fluorescence intensities (FI) were measured by a Varian Cary Eclipse Fluorescence Spectrophotometer (Walnut Creek, CA) at a slit opening of 10 nm and wavelengths of 350 nm (excitation) and 525 nm (emission). FI values were plotted against protein concentrations to determine S_0, which corresponded to the initial slope of the graph as calculated by linear regression.

2.6.3. *Foaming properties*

Foam capacity and stability of samples (10 mg protein/mL) were determined at neutral pH and at the pH where protein solubility was greatest by following exactly the procedure described by Myers et al. (1994). Foam capacity was the volume (mL) of foam produced in 1 min. Foam stability was expressed as the % foam remaining after standing for 15 min.

2.6.4. *Emulsifying properties*

Emulsifying properties were determined according to the method of Wu et al. (1998). Samples were weighed out to provide 1 mg protein/mL and dispersed in 0.01 M phosphate buffer (pH 7) or 0.0249 M $NaHCO_3$·Na_2CO_3 buffer (pH 10). Small amounts of 0.1 M NaOH were added to attain a final sample pH of 7.0 or 10.0. Mixtures were allowed to stand for 15 min. Emulsions were prepared by homogenizing mixtures of 6 mL sample supernatants and 2 mL corn oil with a hand-held homogenizer operated at high setting (20,000 rpm) for 1 min. Emulsification activity index (EAI, in m^2/g) and emulsion stability index (ESI, in min) were calculated from absorbance readings taken at 500 nm.

2.6.5. *Water-holding capacity (WHC)*

WHC of the samples was determined by adapting the method of Balmaceda et al. (1984). We modified the method in the following ways: capped tubes with samples were placed on a platform shaker for 15 min instead of being stirred individually, sample pH was adjusted to 7.0 or 10.0 by adding 0.1 M NaOH, and a water-bath set at 60 °C was used for heating instead of a hot plate. All other steps and calculations were done exactly as described in the original method.

2.7. *Statistical analyses*

Statistical analyses were performed by using the SAS® Systems for Windows software (SAS Institute Inc., Cary, NC). Analysis of variance and Bonferroni *t*-tests or Duncan's multiple range tests were performed on duplicate replications of data to determine significant differences among the treatments ($p \leq 0.05$).

3. Results and discussion

3.1. *Moisture, oil and protein contents*

The milkweed seed contained 1.8% moisture, 20.8% (dry basis, db) crude oil, and 30.4% (db) crude protein. The oil and protein contents were similar to those reported earlier by Evangelista (2007). Oil content of undefatted milkweed seed is about the same as those of cottonseed and soybean, while its protein content is similar to those of peanut kernel and sunflower seed (Wolf, 1983). After defatting with hexane, the oil content of the meal was 0.4% (db) and crude protein content was 44.4% (db).

3.2. *Soluble classes*

Water-soluble (albumin) and NaCl-soluble (globulin) proteins were the predominant fractions in milkweed seed protein, accounting for 22.3% and 15.5%, respectively, of total protein. The ethanol-soluble fraction (prolamin) was 12.2% of the total protein amount and the NaOH-soluble fraction (glutelin) was the least at 3.5%. A substantial amount of protein, 46.5%, remained unextracted and its properties are unknown at this time. It may be possible to reduce further the amount of residual protein by including an additional extraction step using 60% acetic acid to recover acid-soluble proteins, as was done in the original method of Hu and Esen (1981). The distribution of soluble protein classes in milkweed seed is markedly different from those determined by Nikokyris and Kandylis (1997) for high-protein seeds (92% albumins + globulins, 7% glutelins, <1% prolamins) and cereals (28% albumins + globulins, 40% glutelins, 33% prolamins).

3.3. *Amino acid composition*

Glutamic acid, arginine, and leucine had the greatest amounts among amino acids in milkweed seed protein (Table 1). The sulfur-containing amino acids, methionine and cysteine, were also present in detectable quantities. Milkweed seed protein was compared with that of soybean flour, which was used successfully as a protein extender in commercial plywood glue (Hojilla-Evangelista, 2002). Milkweed seed protein had less amounts of isoleucine, leucine, lysine, threonine, tyrosine, valine, serine, and, most notably, aspartic acid, but the other amino acids were present in comparable quantities as those in soybean flour.

The water-soluble protein fraction had an amino acid composition that was nearly similar to that of the ground milkweed seed protein (Table 1); however, its lysine, tyrosine, cysteine, and glutamic acid contents were significantly greater, although only the latter two were at levels that surpassed that of soybean flour. The salt-soluble fraction and the residue after sequential extraction both had substantially higher amounts of most of the amino acids (Table 1) and their profiles were closer to that of soybean flour than of milkweed seed protein.

3.4. *Electrophoresis results*

Milkweed seed protein showed 12 bands, with MW ranging from ca. 100 to <6.5 kDa (Fig. 1, lane 2). The most intense and widest bands resolved at 6.5 kDa, between 19 and 50 kDa, and near 96 kDa. The water-soluble fraction showed 8 distinct bands, with MW ranging from ca. 50 to <6.5 kDa (lane 3). The most concentrated bands resolved at 6.5 and 19 kDa. The 6 bands of the salt-soluble fraction were in the MW range of also ca. 50–6.5 kDa (lane 4), with the darkest bands resolving at less than 6.5 kDa and near 36 kDa. The bands for the NaOH-soluble fraction (lane 6) and protein in the spent solids (lane 7) were substantially fewer and lighter-

Table 1 – Amino acid compositions (g/16 g nitrogen) of soybean flour and ground defatted milkweed seeds and fractions

Amino acid	Soybean flour[a]	Ground defatted milkweed seed	Water-soluble fraction	NaCl-soluble fraction	Residue
Histidine[b]	2.7	2.28 b	2.42 ab	2.67 a	2.47 ab
Isoleucine	4.9	3.68 b	3.66 b	4.64 a	4.41 a
Leucine	8.0	5.81 b	6.00 b	6.71 a	7.29 a
Lysine	6.4	4.44 b	5.28 a	4.82 ab	4.66 ab
Methionine	1.4	1.35 b	1.39 b	1.36 b	1.66 a
Phenylalanine	5.3	4.62 c	4.47 c	6.44 a	5.36 b
Threonine	4.2	2.34 b	2.22 b	2.31 b	3.86 a
Tryptophan	1.2	1.14 b	1.05 b	1.85 a	0.96 b
Tyrosine	3.9	2.18 d	2.39 c	2.53 b	2.66 a
Valine[b]	5.3	4.24 b	4.16 b	5.36 a	5.32 a
Alanine	4.0	3.55 b	3.45 b	3.93 b	5.12 a
Arginine	7.0	8.65 c	9.94 b	11.34 a	7.37 d
Aspartic acid	11.3	6.95 c	6.38 d	8.52 a	7.36 b
Cysteine	1.6	1.66 b	2.21 a	1.16 c	0.94 c
Glutamic acid	17.2	18.14 c	23.58 a	20.62 b	14.61 d
Glycine	4.0	4.42 b	5.02 ab	5.10 ab	5.54 a
Proline	4.7	3.51 b	3.82 b	4.56 a	3.81 b
Hydroxyproline	No data	0.44 b	0.06 c	0.02 c	2.36 a
Serine	5.0	3.04 b	3.30 ab	3.38 ab	4.20 a

Values are means of duplicate determinations. Means across columns followed by different letters are significantly different ($p < 0.05$).

[a] Data from Perkins (1995).

[b] Histidine through valine are essential amino acids.

colored than those observed for whole seeds, water-soluble fraction and salt-soluble fraction. There were no bands that resolved at all from the ethanol-soluble fraction. A more concentrated starting material and/or longer dialysis in the preparation may be needed for the bands to be visible after electrophoresis.

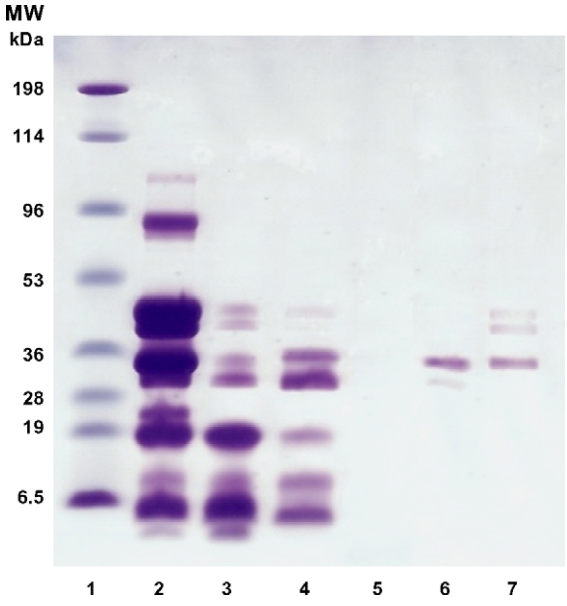

Fig. 1 – SDS-PAGE band patterns of proteins in milkweed seed: (1) MW standards; (2) ground, defatted seed; (3) water-soluble extract; (4) NaCl-soluble extract; (5) ethanol-soluble extract; (6) NaOH-soluble extract; and (7) residue after protein extraction. Concentration = 4 mg protein/mL; sample load volume = 15 μL.

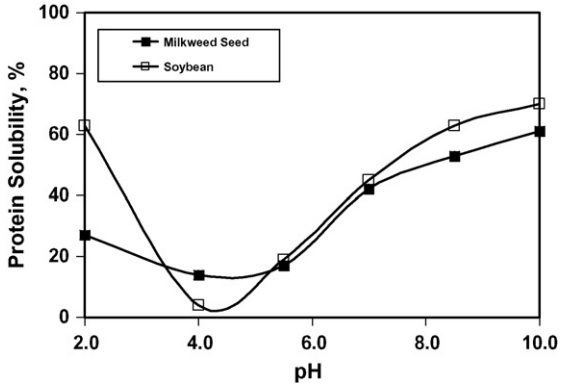

Fig. 2 – Protein solubility profiles of ground, defatted milkweed seed and soybean.

3.5. *Protein solubility*

Milkweed seed protein showed a sigmoidal solubility profile similar to that of soybean protein from pH 4.0 to 10.0 (Fig. 2). Milkweed seed protein was least soluble (12%) at pH 4, had 40% solubility at pH 7, and was most soluble (60%) at pH 10. The presence of alkali generally improves protein solubility by causing dissociation and disaggregation of proteins; however, high solubility at alkaline pH may also be caused by extensive proteolysis (Kinsella, 1976).

The solubility profile of a protein is a practical indicator of its denaturation and also gauges the potential or limitation of the protein as a functional ingredient because it is related to other properties, such as foaming, emulsification, and gelation (Kinsella, 1976). Milkweed protein showed substantial solubility at both pH 7 and 10, which indicated potential applications

Table 2 – Functional properties of ground milkweed seeds at pH 7 and 10

Sample and pH	Functional properties[a]					
	S_0	FC (mL)	FS % foam left	EAI (m^2/g)	ESI (min)	WHC (g water/g protein)
Milkweed seed, pH 7	165 ± 5 a	101 ± 10 b	39.8 ± 4.0 a	102.7 ± 3.8 b	15.4 ± 0.3 a	3.70 ± 0.02 a
Milkweed seed, pH 10	128 ± 2 b	116 ± 6 a	4.4 ± 1.5 b	248.8 ± 11.9 a	14.3 ± 0.1 a	2.91 ± 0.13 a

Values are means ± standard deviations of duplicate determinations. Means within a column followed by different letters are significantly different ($p < 0.05$).

[a] S_0: surface hydrophobicity index; FC: foaming capacity; FS: foam stability; EAI: emulsion activity index; ESI: emulsion stability index; WHC: water-holding capacity.

in aqueous systems and high-alkaline environments, such as plywood glues. Thus, the other functional properties were evaluated at both neutral pH and 10.

3.6. *Surface hydrophobicity index (S_0)*

S_0 indicates the extent of exposure of hydrophobic regions of protein molecules; higher values suggest more unaggregated proteins. The S_0 values for milkweed seed protein at pH 7 and 10 (Table 2) were significantly lower than the 530 we determined for soybean protein (Hojilla-Evangelista et al., 2004). This result suggests that at neutral and alkaline pH, milkweed seed are poorly dispersed, causing restricted access to hydrophobic regions and promoting aggregation. The markedly lower S_0 values implied a substantial presence of aggregated proteins in milkweed seed proteins.

3.7. *Foaming properties*

Milkweed seed protein produced more than 100 mL of foam at pH 7 and 10 (Table 2), but these volumes were still less than the 135 mL of foam generated by the same concentration of soybean protein at neutral pH (Hojilla-Evangelista et al., 2004). Foam stability was rated fair at pH 7, where only 40% of the foam initially generated remained after 15 min of standing. At pH 10, the foam collapsed soon after being formed. The foam produced by milkweed seed protein was considerably less stable than that produced by soybean protein, which was reported to retain 95% of its foam at the end of the allotted standing time (Hojilla-Evangelista et al., 2004). It may be that milkweed seed has limited availability of proteins (as supported by the low S_0 values) that can participate in interfacial interactions, thus resulting in less-stable foams.

3.8. *Emulsifying properties*

Higher values for the emulsion activity index (EAI) generally indicate better emulsifying capacity. At pH 7, the protein from milkweed seed showed excellent emulsifying capacity, as supported by an EAI value (Table 2) that was double that of soybean protein (56 m^2/g protein) (Hojilla-Evangelista et al., 2004). The emulsifying capacity of milkweed seed protein was even far superior at pH 10 than at pH 7 (Table 2). Some treatments, such as heating and limited hydrolysis, can result in improved emulsifying capacity if the protein structure can unfold without resulting in aggregation (Cheftel et al., 1985). We hypothesize that the marked increase in EAI for the protein prepared at pH 10 may have been due to higher quantities of soluble proteins generated by hydrolysis under alkaline conditions.

Emulsion stability index (ESI) values at pH 7 and 10 were nearly identical (Table 2), implying that the stability of the emulsion formed by milkweed seed protein was not affected by the change from neutral to alkaline pH. ESI for milkweed seed protein was the same as that reported for soybean protein (15.0 min) (Hojilla-Evangelista et al., 2004). Xu and Diosady (1994) reported that emulsion stabilities among proteins from soybean, canola, and rapeseed were similar.

3.9. *Water-holding capacity (WHC)*

WHC of a protein plays an important role in imparting characteristics such as texture, body, viscosity, and adhesion (Cheftel et al., 1985). WHC values for milkweed seed protein were nearly the same at the two pHs used (Table 2). Both were notably less than the 6.7 g water/g protein we observed for soybean protein at neutral pH (Hojilla-Evangelista, unpublished data) but nearly equal to the 3.4 g water/g protein we reported for *Cuphea* protein at pH 10 (Hojilla-Evangelista and Evangelista, 2006). Conditions that cause protein denaturation and aggregation generally result in decreased WHC because of the reduction of protein surface area and availability of polar amino groups for hydrogen bonding with water molecules (Cheftel et al., 1985). The relatively low S_0 values we obtained (Table 2) indicated a notable presence of aggregated proteins in milkweed seed proteins. This may explain the unremarkable WHC values for milkweed seed protein.

4. Conclusions

Milkweed seed protein had significant functional properties at pH 7 and 10. More than 40% of the protein was soluble in the pH range of 7–10. The protein produced substantial foam volumes, but the foam was not stable. Its emulsifying capacity was excellent and emulsions formed were stable. These results showed that milkweed seed protein has functional properties that may find use as a thickener, protein extender in adhesives, or emulsifier in paints.

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REFERENCES

AOCS, 1998. Official Methods and Recommended Practices of the American Oil Chemists' Society, 5th ed. AOCS Press, Champaign, IL.

Balmaceda, E.A., Kim, M.K., Franzen, R., Mardones, B., Lugay, J.C., 1984. Protein functionality methodology—standard tests. In: Regenstein, J.M., Regenstein, C.E. (Eds.), Food Protein Chemistry. Academic Press, NY, pp. 278–291.

Cheftel, J.C., Cuq, J.L., Lorient, D., 1985. Amino acids, peptides, and proteins. In: Fennema, O.R. (Ed.), Food Chemistry, 2nd ed. Marcel Dekker Inc., NY, pp. 275, 286, 298–308, 351–355.

Crews, P.C., Sievert, S.A., Woeppel, L.T., McCullough, E.A., 1991. Evaluation of milkweed floss as an insulative fill material. Text. Res. J. 61, 203–210.

Evangelista, R.L., 2007. Milkweed seed wing removal to improve oil extraction. Ind. Crops Prod. 25, 210–217.

Harry-O'Kuru, R.E., Mojtahedi, H., Vaughn, S.F., Dowd, P.F., Holser, R.A., Abbott, T.P., 1999. Milkweed seedmeal: a control for *Meloidogyne chitwoodi* on potatoes. Ind. Crops Prod. 9, 145–150.

Hojilla-Evangelista, M.P., 2002. Adhesive qualities of soybean protein-based foamed plywood glues. J. Am. Oil Chem. Soc. 79, 1145–1149.

Hojilla-Evangelista, M.P., Evangelista, R.L., 2006. Effects of cooking and screw-pressing on functional properties of *Cuphea* PSR23 seed proteins. J. Am. Oil Chem. Soc. 83, 713–718.

Hojilla-Evangelista, M.P., Sessa, D.J., Mohamed, A., 2004. Functional properties of soybean and lupin protein concentrates produced by ultrafiltration–diafiltration. J. Am. Oil Chem. Soc. 81, 1153–1157.

Holser, R.A., 2003. Properties of refined milkweed press oil. Ind. Crops Prod. 18, 133–138.

Hu, B., Esen, A., 1981. Heterogeneity of soybean seed proteins: one-dimensional electrophoretic profiles of six different solubility fractions. J. Agric. Food Chem. 29, 497–501.

Kinsella, J.E., 1976. Functional properties of proteins in foods: a survey. Crit. Rev. Food Sci. Nutr. 7, 219–280.

Knudsen, H.D., Zeller, R.D., 1993. The milkweed business. In: Janick, J., Simon, J.E. (Eds.), New Crops. Wiley, NY, pp. 422–428.

Malcolm, S.B., Zalucki, M.P., 1999. Plant latex and first-instar monarch larval growth and survival on three North American milkweed species. J. Chem. Ecol. 25, 1827–1842.

Myers, D.J., Hojilla-Evangelista, M.P., Johnson, L.A., 1994. Functional properties of protein extracted from flaked, defatted, whole corn by ethanol/alkali during sequential extraction processing. J. Am. Oil Chem. Soc. 71, 1201–1204.

Nikokyris, P.N., Kandylis, K., 1997. Feed protein fractions in various solvents of ruminant feedstuffs. J. Sci. Food Agric. 75, 198–204.

Perkins, E.G., 1995. Composition of soybeans and soybean products. In: Erickson, D.R. (Ed.), Practical Handbook of Soybean Processing and Utilization. AOCS Press, Champaign, IL, and United Soybean Board, St. Louis, MO, p. 16.

Sorgentini, D.A., Wagner, J.R., Añon, M.C., 1995. Effects of thermal treatment of soy protein isolate on the characteristics and structure-function relationship of soluble and insoluble fractions. J. Agric. Food Chem. 43, 2471–2479.

Wolf, W.J., 1983. Soybeans and other oilseeds. In: Kirk-Othmer (Ed.), Encyclopedia of Chemical Technology, 21, 3rd ed. John Wiley and Sons, Inc., NY, pp. 417–442.

Wu, W.U., Hettiarachchy, N.S., Qi, M., 1998. Hydrophobicity, solubility, and emulsifying properties of soy protein peptides prepared by papain modification and ultrafiltration. J. Am. Oil Chem. Soc. 75, 845–850.

Wu, Y.V., Hojilla-Evangelista, M.P., 2005. *Lesquerella fendleri* protein fractionation and characterization. J. Am. Oil Chem. Soc. 82, 53–56.

Xu, L., Diosady, L.L., 1994. Functional properties of Chinese rapeseed protein isolates. J. Food Sci. 59, 1127–1130.